REPORT ON STANDARDIZATION OF MICROCHEMICAL METHODS

By C. O. WILLITS, *Referee*, and C. L. OGG, *Associate Referee* (Eastern Regional Research Laboratory,[1] Philadelphia 18, Pennsylvania)

COLLABORATIVE STUDIES ON CARBON AND HYDROGEN

In 1948, two samples were analyzed for carbon and hydrogen at 20 different laboratories. A statistical study of the results was reported at the annual A.O.A.C. meeting in 1948 (8), and a further study of the data was presented at the American Chemical Society, Atlantic City meeting, in 1949. In this study, each collaborator analyzed the two samples, following the procedure currently used by him. The methods were all similar in principle, but no two employed the same combination of techniques for the different steps involved. To determine which techniques produced the best results, statistical studies of the results were made. These indicated that larger weight samples, not wiping the absorption tubes, and not replacing the oxygen in the absorption tubes with air produced more accurate results. The statistical studies also showed that the other common variations in the procedures had little if any effect on the results, for example, values obtained by using mechanically operated, electrically heated sample burners did not differ statistically from those obtained by manually operated gas burners.

A method for carbon and hydrogen analysis based on the results of these studies was submitted to the 1950 collaborators. The method employed the techniques which appeared to produce the best results for the three steps found critical in 1948, plus the techniques for the other operations which resulted in the simplest, most easily performed procedure.

The method, described below, and samples of the same two materials analyzed in 1948, nicotinic acid and benzyl-iso-thiourea hydrochloride, were sent to the 1950 collaborators.

CARBON AND HYDROGEN DETERMINATION

REAGENTS

Copper oxide.—Wire form, about 1 mm in diam. and 3–4 mm long; discard material finer than 20 mesh. Ignite at 800–900°C. for 1 hr before placing in combustion tube.

Platinum gauze, 52 mesh.—From three 3×5-cm sections, make 3 rolls, 30 mm long × 7 mm O.D. Boil in 1-1 nitric acid for 15 min and ignite in nonluminous Bunsen burner flame.

Asbestos.—Gooch crucible asbestos; ignite at 800–900°C. for 30 min and store in wide mouth bottles.

Silver.—Fine wire or ribbon; if tarnished reduce in stream of hydrogen at 350–450°C.

Lead dioxide.—Pellets, 1–2-mm diam., special grade for micro analysis; or prepare by digesting commercial grade powder in concentrated nitric acid for 2 hrs, let stand for 1 hr, decant nitric acid, wash with distilled water until free of nitric acid, evaporate to dryness, and cut into 2-mm cubes. Roll cubes in jar to round corners and sieve out powder.

Glass wool.—Pyrex, pliable.
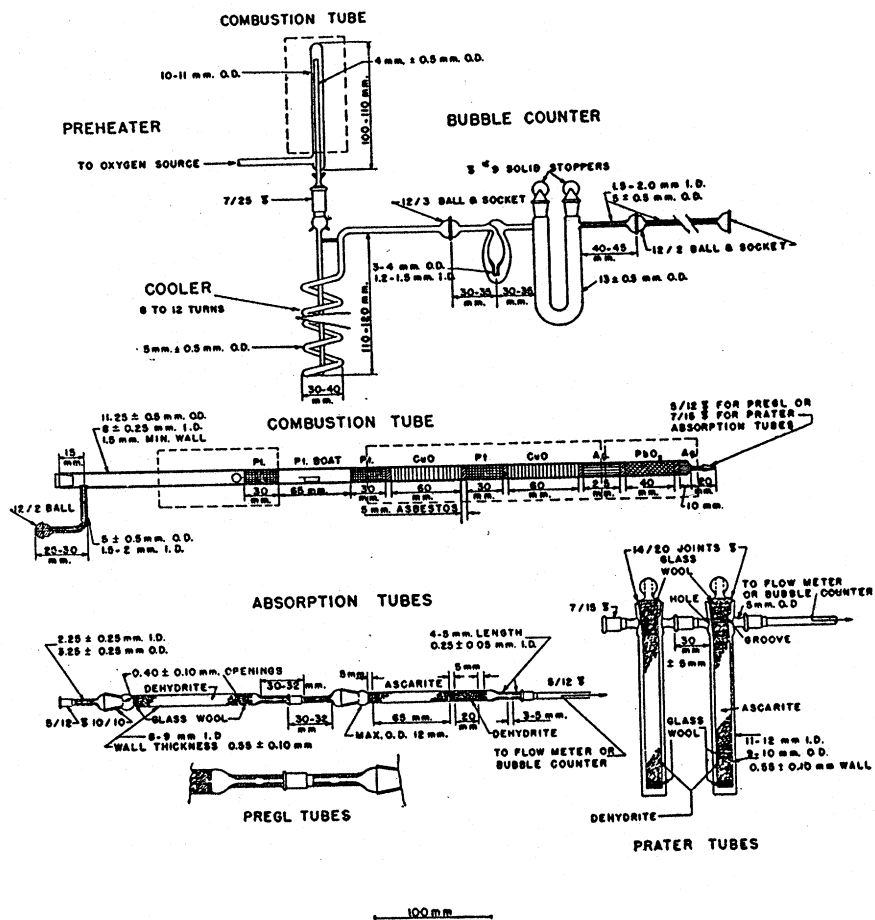
CARBON & HYDROGEN APPARATUS

Fig. 1

Dehydrite or Anhydrone.—(Magnesium perchlorate, anhydrous.) Break pieces to less than 3 mm long; discard portion passing 40-mesh sieve.

Ascarite.—(Sodium hydroxide on asbestos.) Use commercial preparation of 8–20 mesh.

Oxygen.—Cylinder with pressure regulator adjustable from 0–10 lb pressure on the low-pressure side and with needle-valve control.

Preheater.—As specified by American Chemical Society Committee on Stand-

ardization of Microchemical Apparatus[2] except with 12/2 ball joint.[3]

Bubble counter and U-tube.—According to a A.C.S. specification except with ball joints.[3]

Combustion tube.—Fused quartz (or Vycor) glass,[4] dimensions as per A.C.S. specifications[5] but with 12/2 ball joint[3] on side arm and 5/12 or 7/15 inner joint[3] on exit end.

Absorption tubes.—Pregl type, as per A.C.S. specification, but with 5/12 joints[3] (alternative, Prater type, semimicro size with 7/15 joints[3]).

Bubble counter or flowmeter.—Any convenient arrangement which will measure 10–30 ml /min flow of gas from exit end of second absorption tube.

Preheater furnace.—Electric[6,7] 12–14 mm I.D. by 5 in. long; maintain at temp. of $600 \pm 25°C$.

Burning furnace.—Electric[6,7] 13–14 mm I.D. by 4 in. long. Furnace should reach a temp. of 600–700°C. in 5 min, about 800°C. in 15 min, with max of 850°C. in 30 min.

Long furnace.—Electric[6,7] 13–14 mm I.D. by 8 in. long; maintained at a temp. of 775–800°C.

Constant temperature mortar.—Electric[6] 13–14 mm I.D. by 3 in. long, thermostatically controlled to maintain a temp. of $177 \pm 2°C$.

Boat.—Platinum, micro.

Finger cots.—Chamois.

Tweezers.—Platinum tipped.

PREPARATION OF APPARATUS

Preheater.—Place copper oxide in preheater tube, connect spiral cooling coil, immerse coil in beaker of water, and support assembly by suitable clamps and stand. Place electric furnace over preheater tube and maintain at ca 600°C. Connect side arm of combustion unit to needle valve of oxygen pressure regulator by suitable tubing rubber or tygon.

Bubble counter-U-tube.—Fill bubble counter and U-tube by placing glass wool plug at bottom of U, fill side next to bubble counter with Dehydrite to within ½ in. of side arm and cap with another glass wool plug. Place Ascarite layer in other side to within 1½ in. of side arm, then insert a glass wool plug, ca 1 in. of Dehydrite, and finally a second plug. Cement in stoppers with glass cement or paraffin, then with medicine dropper introduce concentrated sulfuric acid into bubbler until level is 3–4 mm above bubbler tip. Connect to preheater with pressure clamp.

Combustion tube.—Clean and dry combustion tube (Fig. 1). Place 10-mm roll of silver in exit end with one or two strands reaching to open end of ground joint. Insert a loose asbestos plug (not choking plug), 40 mm of lead dioxide, asbestos plug, and a second silver roll 25 mm long; which should extend into long furnace about ½ inch. Introduce asbestos plug, 60 mm of copper oxide, asbestos plug, 30-mm platinum gauze roll, asbestos plug, 60-mm copper oxide, asbestos plug, and finally 30-mm platinum gauze, which should extend about 10 mm beyond end of long furnace. Place prepared tube in furnaces with exit end protruding beyond constant-temp. mortar sufficient to permit connecting absorption tubes. Connect side arm to bubble counter-U-tube.

Absorption tube.—Place glass wool plug in end of water absorption tube, fill tube to within ½ in. of other end with Dehydrite or Anhydrone, and cap with second glass

2 *Analytical Chemistry*, 21, 1555 (1949).
3 Rubber connectors may be used.
4 If Pyrex tubes are used, furnace temperatures should not exceed 725°C.
5 10 or even 12 mm I.D. tubes may be used, but oxygen flow rate must be increased proportionately.
6 Gas heaters may be used but specified temperatures should be maintained.
7 Temperature of furnaces measured at center of furnace inside empty combustion tube having one end stoppered.

wool plug. If Pregl tubes are used, seal ground-glass joint with enough glass cement to give clear seal and remove any excess on outer surface of tube with cotton dipped in benzene or other solvent. If Prater tubes are used, lubricate lower ¾ of inner joint with minimum of light stopcock grease and insert in outer tube. Prepare carbon dioxide absorption tube by placing glass-wool plug in end, and fill tube to about 1½ in. of other end with Ascarite. Insert ¼ inch glass-wool plug, add ¼-inch layer of Dehydrite, and cap filling with another glass-wool plug. Complete assembly of absorption tube as directed for water absorption tube. Connect absorption tubes to combustion tube with ground joints (use no lubricant) or with special impregnated rubber tubing.

Attach bubble counter or flowmeter to exit end of carbon dioxide absorption tube. Counter or meter must be calibrated so that flow rate can be set at 15 to 20 ml per min.

DETERMINATION

Conditioning apparatus.—After various parts of apparatus have been prepared and assembled, condition combustion tube for 3–4 hr with long furnace at 775–800°C. and with oxygen flowing thru apparatus at rate of 15–20 ml per min.[8] At the same time, make two simulated sample burnings, without sample, with burning furnace at 825–850°C. (Temp. must be ca 100°C. lower if Pyrex combustion tubes are used.)

Burn an unweighed 10–15-mg sample to condition combustion and absorption tubes. With absorption tubes connected, adjust needle valve on pressure regulator so that oxygen flow is 15–20 ml per min., and place burning furnace about 3 in. from. long furnace. Place platinum boat containing sample in combustion tube ca 2 in. from long furnace. Insert platinum flashback roll (Fig. 1) so that end of gauze is even with face of furnace next to sample, and stopper tube. Turn on burning furnace and allow it to reach temp. of ca 600°C. before starting sample combustion by moving furnace over sample at rate of 1 in. in 6–8 min. Move the burning furnace across sample only once, taking 18–24 min for full travel of furnace. Turn off burning furnace 5 min after it reaches long furnace but continue to sweep oxygen thru tube for an additional 15 min. before disconnecting absorption tubes. Remove absorption tubes and place by balance to equilibrate. Handle tubes only with clean, chamois finger cots. If Prater tubes are used, turn joints ¼-turn to seal. If rubber connections are used, wipe only tips of tubes with moist, then dry, chamois before placing them by balance. Wait 10 min. if ground joints were used or 15 min if rubber connections were made, then weigh carbon dioxide-absorption tube first and water-absorption tube second. A glass tare with a volume (surface) ca equal to that of absorption tubes should be used when weighing tubes. Record weights of tubes and then reconnect tubes to combustion tube for subsequent analysis.

Proving the apparatus.—Replace boat with one containing 10–15-mg sample of your own standard compound weighed to nearest 0.01 mg. Repeat combustion and weighing procedure described above. Calculate percentage of carbon and hydrogen in standard sample from increase in weight of carbon-dioxide and water-absorption tubes. Repeat analysis until results from two consecutive runs are within 0.30 per cent of theoretical values and means of carbon and hydrogen results are within 0.20 per cent of theoretical value for the standard compound.

When apparatus has met this test, proceed with analysis of sample, using procedure described above. (Humidity conditions of room may make it necessary to correct apparent weight of water by subtracting a blank value.)

---

[8] Use 3–4 lb. oxygen pressure head on low pressure side of pressure regulator.

TABLE 1.—*Statistical summary of carbon values*

| COLLABORATOR NO. | NICOTINIC ACID | | | | | | BENZYL-ISO-THIOUREA HYDROCHLORIDE | | | | | |
| | 1948 | | | 1950 | | | 1948 | | | 1950 | | |
	n	$\bar{x}$ %C	s	n	$\bar{x}$ %C	s	n	$\bar{x}$ %C	s	n	$\bar{x}$ %C	s
0	8	58.47	0.204	9	58.59	0.069	8	47.38	0.044	11	47.42	0.061
2	4	58.44	.173	2	58.62	.050	3	47.32	.087	3	47.49	.044
8	8	58.75	.217				8	47.43	.246			
10	2	58.75	.092				2	47.72	.022			
12	3	58.99	.056				3	47.68	.026			
13												
17	8	58.64	.145	5	58.49	.111	2	47.44	.057	6	47.48	.142
23	3	58.53	.108	3	58.85	.076	4	47.42	.179	3	47.72	.087
24	6	58.64	.072				6	47.51	.123			
27	3	58.57	.071				3	47.48	.056			
28	3	58.61	.229				3	47.39	.216			
31	2	58.40	.020				3	47.55	.199			
35	8	59.14	.869	4	58.47	.106	5	47.51	.278	4	47.43	.014
37				4	58.68	.096				4	47.44	.160
39	4	58.70	.226	6	58.53	.110	4	47.42	.184	8	47.32	.133
40	4	58.62	.060	3	58.54	.066	4	47.58	.053	3	47.34	.022
41	5	58.92	.078				4	47.66	.103			
44	4	58.73	.099	4	58.75	.082	3	47.14	.061	3	47.28	.187
45	8	58.64	.189	5	58.66	.099	10	47.59	.167	5	47.52	.113
46	6	58.66	.123	3	58.75	.149	3	47.55	.142	2	47.82	.113
49	21	58.73	.326				17	47.55	.145			
50	8	58.67	.118	8	58.72	.081	3	47.53	.195	7	47.69	.115
Mean		58.68	.174		58.64	.091		47.49	.129		47.50	.099
Mean*		58.68	.214		58.64	.095		47.46	.136		47.50	.099
Theoretical value		58.53			58.53			47.40			47.40	
t calculated		0.69						0.19				

* Values for data obtained by the 10 collaborators who participated in both studies.

Twelve laboratories reported 55 carbon and hydrogen values for sample 1 (nicotinic acid) and 59 values for sample 2 (benzyl-iso-thiourea hydrochloride). Table 1 shows the number of carbon values reported, n, the mean, $\overline{X}$, and standard deviation, S, for each analyst's data. Similar data for the 1948 results are also included.

This method of presenting the data was chosen instead of the histograms used in previous reports because it summarizes the data in a condensed but complete form. The data so presented make it easy to compare the relative accuracy and precision of the results for each sample by each analyst and by all the analysts. Further, since all the essential data are presented, the work of any succeeding collaborative study can be readily compared.

Although inspection showed generally close agreement between the two sets of data, a statistical comparison was made to test the relative accuracy. The mean of the $\overline{X}$'s for each year was determined for each sample and Student's t test (5) applied to determine whether or not the two means were significantly different.

$$t = \bar{x}\sqrt{\frac{n_a n_b (n_a + n_b - 2)}{(n_a + n_b)Sx^2}}$$

($\bar{x}$ is the difference between the two means, n_a and n_b the number of $\overline{X}$'s for 1948 and 1950, respectively, and Sx^2 is the pooled sum of squares.)

The t values obtained, 0.69 for carbon on sample 1 and 0.19 for sample 2, show little indication of any actual difference between the means. The greatest difference between the two sets of data is the lower and more uniform standard deviations obtained in 1950. The means of the $\overline{X}$'s and S's obtained by the 10 collaborators who participated in both the 1948 and 1950 studies differed only slightly from those for the total data.

Table 2 shows a summary of the hydrogen data obtained in 1948 and 1950, presented in a manner similar to that used for carbon in Table 1. Comparison of the means of the individual $\overline{X}$ values shows that the 1950 values are nearer the theoretical values of 4.09 and 5.47 per cent H for samples 1 and 2, respectively, than those obtained in 1948. The t test was again applied to determine the significance of the difference between the mean values. The t value for sample 1 was 0.99, which indicates a probability of 0.67 (67 chances in 100) that the difference between the 1948 and 1950 means was not due to chance alone. For sample 2, t was 1.62, and the probability 0.88 that the difference between the means was real. In 1948, the average standard deviations for the two samples were 0.095 and 0.119, whereas in 1950, slightly lower values—0.087 and 0.092—were obtained.

When the mean values for the ten analysts who participated both years were compared, the only difference of any significance was between the

TABLE 2.—*Statistical summary of hydrogen values*

COLLABORATOR NO.	NICOTINIC ACID						BENZYL-ISO-THIOUREA HYDROCHLORIDE					
	1948			1950			1948			1950		
	n	$\bar{x}$ %H	s	n	$\bar{x}$ %H	s	n	$\bar{x}$ %H	s	n	$\bar{x}$ %H	s
0	8	4.10	0.152	9	4.10	0.052	8	5.44	0.161	11	5.43	0.151
2	4	4.21	.098	2	4.07	.022	3	5.57	.107	3	5.49	.052
8	8	4.68	.123				8	5.90	.140			
10	2	4.10	.010				2	5.48	.036			
12	3	3.98	.052				3	5.35	.173			
13												
17	8	4.27	.089	5	4.01	.158	2	5.69	.240	6	5.35	.124
23	3	3.97	.110	3	4.05	.091	4	5.41	.180	3	5.53	.077
24	6	4.16	.078				6	5.56	.045			
27	3	4.09	.052				3	5.44	.041			
28	3	4.04	.121				3	5.46	.160			
31	2	4.06	.099				3	5.37	.156			
35	8	4.22	.143	4	4.20	.062	5	5.51	.136	4	5.55	.056
37				4	4.09	.050				4	5.41	.042
39	4	3.95	.090	6	4.17	.056	4	5.35	.096	8	5.49	.067
40	4	4.09	.085	3	4.08	.056	4	5.48	.107	3	5.53	.051
41	5	4.37	.099				4	5.77	.085			
44	4	4.20	.052	4	4.02	.101	3	5.53	.052	3	5.47	.020
45	8	4.17	.065	5	4.23	.035	10	5.56	.092	5	5.62	.062
46	6	4.32	.075	3	4.17	.274	3	5.68	.115	2	5.17	.255
49	21	4.12	.186	8	4.17	.090	17	5.42	.205	7	5.40	.148
50	8	4.11	.111				3	5.61	.048			
Mean		4.16	.095		4.11	.087		5.53	.119		5.45	.092
Mean*		4.14	.097		4.12	.098		5.53	.123		5.45	.101
Theoretical value		4.09			4.09			5.47			5.47	
t. calculated				0.99						1.62		

* Values for data obtained by 10 collaborators who participated in both studies.

TABLE 3.—*Statistical comparison of carbon and hydrogen values*

YEAR	NICOTINIC ACID						BENZYL-ISO-THIOUREA HYDROCHLORIDE					
	CARBON			HYDROGEN			CARBON			HYDROGEN		
	n	$\bar{X}$ %C	s	n	$\bar{X}$ %H	s	n	$\bar{X}$ %C	s	n	$\bar{X}$ %H	s
1948	101	58.66	0.219	101	4.18	0.208	92	47.51	0.184	92	5.53	0.199
1950	55	58.63	.136	55	4.12	.115	59	47.48	.174	59	5.46	.131
$2S_{\bar{x}}$		0.057			0.052			0.059			0.054	
$\bar{X}(1948)-\bar{X}(1950)$		0.03			0.06			0.03			0.07	
F, calculated		2.59			3.27			1.12			2.31	
F, critical (98%)		1.78			1.78			1.75			1.75	
Theoretical value		58.53			4.09			47.40			5.47	

mean of the $\overline{X}$'s for sample 2. The t value for these two sets of data was 1.58, and the probability of the difference being real was 0.88.

A second type of comparison was made in which the total data for each element for each compound were considered as a representative population sample. Table 3 shows the mean and standard deviation for each set of data for 1948 and 1950.

The relative accuracy of the 1948 and 1950 data was determined (1) by calculating the standard deviation of the difference between means.

$$S_{\overline{x}} = \sqrt{\frac{S_a^2}{n_a} + \frac{S_b^2}{n_b}}$$

and comparing 2 times this value ($2S_{\overline{x}}$) with the numerical difference between means ($\overline{X}_a - \overline{X}_b$). If the latter value was the greater, as was true for hydrogen for both samples, the difference between means was significant at the 95 per cent level.

Snedecor's F test (6) was used to compare the precisions obtained in the two studies. In three of the four cases, the precision obtained in 1950 was significantly better at the 98 per cent level than that in 1948; only for carbon in sample 2 was there no definite improvement in precision.

Even though the analysts were unfamiliar with the new method, the results obtained were superior to or as good as those obtained when each analyst used his own, with which he was completely familiar. This indicated that the method has excellent possibilities of being suitable for adoption by the A.O.A.C. The Referees therefore recommend that the 1950 test method for carbon and hydrogen be adopted, first action, and that further work be done to prove the method through the analysis of a greater variety of materials and to look for possible improvements in the procedure.

MICROKJELDAHL DETERMINATION OF NITROGEN

The 1949 collaborative studies on determination of nitrogen by the Kjeldahl procedure resulted in the adoption as first action of the method employed in those studies. In keeping with the recommendation that further studies be made both on the regular method and on a method for determining N—O and N—N bonded nitrogen, investigation of the method was continued this year.

A. *Effect of Potassium Sulfate Concentration.*—It had been suggested that better results would be obtained by changing the high potassium sulfate concentration of the digestion mixture from the recommended 1.30 g per 2 ml of sulfuric acid to a lower value. Consequently, the 1950 collaborators were asked to analyze a sample of the same nicotinic acid used in the 1949 studies by the same method (9), with the one exception that only 0.85 g of potassium sulfate be used per 2 ml of sulfuric acid.

Table 4 shows the 77 results obtained by the 14 analysts reporting. The number of determinations, n, the mean, $\overline{X}$, and the standard deviation, S, are given for each analyst. For ease of comparison, the same data for each collaborator participating in 1949 are presented. Although inspection of the data shows conclusively that better results were obtained with the

TABLE 4.—*Statistical summary of Kjeldahl nitrogen values for nicotinic acid*

COLLABORATOR NO.	1949 (1.30 g K_2SO_4)			1950 (0.85 g K_2SO_4)		
	n	$\overline{X}$ (% N)	s	n	$\overline{X}$ (% N)	s
0	13	11.37	0.043	4	11.14	0.085
6				2	11.02	.106
9	8	11.35	.045	8	11.33	.059
15	5	11.60	.190	6	10.48	1.01
19				9	6.32	2.00
21				8	11.24	.076
22	2	11.34	.056	2	11.31	.028
23	3	11.35	.280	3	6.14	1.30
24	3	11.30	.020			
25	3	11.33	.125			
29	3	11.34	.044	3	11.33	.030
30	8	11.44	.073	8	11.39	.124
36	3	11.31	.010			
37	8	11.20	.167	2	8.44	2.23
49	5	11.23	.187			
51	9	11.15	.303	8	11.42	.125
57	3	11.41	.050	8	11.24	.092
59				6	11.03	.103
Mean		11.34	.114		10.27	.526
Mean*		11.36	.125		10.42	.508
Theoretical value		11.38			11.38	
t, calculated			2.13			
t*, calculated			1.60			

* Values for data obtained by the 10 collaborators who participated in both studies.

larger amount of potassium sulfate, a statistical comparison was made. As with carbon and hydrogen, Student's t test (5) was used to test the significance of the difference between means. Two separate comparisons were made, one utilizing all the data for both years, and the other with only the data obtained from those collaborators who participated in both studies. The t value obtained for the first comparison was 2.13, with a resulting probability of 0.96 that the difference was not due to chance; for the second comparison, the t value was 1.60, with a probability of 0.87.

The values reported in 1950 were considered as a single population sample and compared with those for 1949. Table 5 shows the results. Critical differences in precision (F value), and accuracy (difference between means) were obtained, the 1949 values being better in both instances. In both types of statistical comparison, therefore, there was strong evidence that the method employing 1.30 g of potassium sulfate gave results superior to those obtained with 0.85 g.

TABLE 5.—*Statistical comparison of nitrogen values*

YEAR	NICOTINIC ACID		
	n	$\overline{X}$ (% N)	s
1949	76	11.17	0.59
1950	78	10.35	1.95
$2\,S_{\overline{x}}$		0.46	
$\overline{X}(1949) - \overline{X}(1950)$		0.82	
F, calculated			10.94
F, critical (98%)			1.71
Theoretical value		11.38	

The means of results obtained by 7 of the 14 analysts who reported in 1950 were within 0.20 per cent of the theoretical value (11.38 per cent), whereas the remaining 7 means were all low by 3 per cent or more. The reason that half the analysts in 1950 failed to obtain satisfactory results while the other half were successful is apparently tied up with the difference in the amount of heat applied to the digestion flasks. Variation in the amount of heat were expected because even similar digestion racks will produce different amounts of heat, depending upon the B.T.U. of the gas or the voltage applied. The temperature of the boiling digestion mixtures was presumably the same in all cases, since each analyst used the same digestion mixture and was instructed to "digest 4 hours at vigorous boil with the acid condensing well up into the neck of the flask." These directions did not specify the amount of heat to be applied because there was no satisfactory way of stating or measuring this. Consequently, the amount of superheating that took place at the glass-liquid interface varied according to the amount of heat applied and is believed to be the cause of the variation in the results obtained. Data published previously (2) have indicated this to be the case. Refractory materials, such as nicotinic acid, fortunately can be quantitatively decomposed if a higher concentration of potassium sulfate (1.3 g per 2 ml sulfuric acid) is used. This eliminates the necessity for extensive superheating, an amount not possible with some digestion rack heaters.

B. *Analyses of Compounds Containing N—O Linkages.*—The failure of the Friedrich-hydriodic acid method for determining N—O and N—N bonded nitrogen to stand up under collaborative test in 1949 led to a search for another method for reducing N—O groups prior to Kjeldahl digestion. The sodium hyposulfite, $Na_2S_2O_4$ (also called sodium hydrosulfite) reduction method of Shaefer and Becker (4) had been slightly modified and used successfully in the Referee's laboratory to analyze compounds not reduced by the salicylic acid or by the carbon methods. As a result, it was decided to test the use of $Na_2S_2O_4$ collaboratively this year. The following directions and two samples, nicotine picrate and p-nitrochlorobenzene, were sent to each collaborator.

Dissolve 10- to 30-mg sample in 3 ml of water, ethanol, or acetone in a 30-ml Kjeldahl flask. Heat if necessary, but cool solution before continuing. Add 0.4 g of sodium hyposulfite ($Na_2S_2O_4$), 3 ml of water, and boil gently for 5 minutes. Cool, add 1 ml of 50 per cent H_2SO_4, place on digestion rack and boil until volume of solution is reduced to approximately 2 ml. Cool, add 1.0 g K_2SO_4, 40 mg HgO, and 1.5 ml H_2SO_4, and continue analysis as directed in the regular microkjeldahl procedure, "First action," beginning with "and digest 4 hours . . . "

Table 6 shows the results obtained for the two samples containing nitro groups. The mean of the means obtained by the different analysts was 17.18 per cent nitrogen for nicotine picrate (theoretical for N, 18.06 per cent). Application of the t test (7) gave a value of 1.87, with a proba-

TABLE 6.—*Statistical summary of nitrogen values by hyposulfite reduction method*

COLLABORATOR NO.	NICOTINE PICRATE			p-NITROCHLOROBENZENE		
	n	$\bar{X}$ %N	s	n	$\bar{X}$ %N	s
0	9	18.07	0.067	10	8.88	0.037
6	2	17.92	.028	2	8.59	.022
9	8	17.78	.330	7	5.40	.888
15	6	17.99	.099	3	8.87	.036
19	8	17.52	.189	8	8.14	.274
21	4	17.33	.182	8	7.81	.313
22	2	17.95	.014	2	8.81	.071
23	3	18.13	.257	3	2.52	.472
29	3	13.22	.218	3	6.12	1.24
30	9	18.09	.078	7	8.81	.107
37	4	17.93	.126	6	6.94	1.67
51	8	17.84	.098	8	7.29	1.20
57	3	17.83	.110	3	8.76	.010
59	6	12.90	.603	—	—	—
Mean		17.18	.171		7.46	.488
Theoretical value		18.06			8.89	
t, calculated		1.87			2.76	

bility of 0.91 that the difference between the mean and theoretical values was not due to chance. This apparently low t value was due to the exceptionally high standard error of the determination. Inspection of the mean values of the 14 analysts showed that two means (collab. No. 29 and 59) were much lower than the others. When these two values were discarded, the mean became 17.87 per cent—only 0.19 per cent—low, but the t value was 2.79 and the probability 0.98 that there was an inherent error in the method.

The mean of the means for p-nitrochlorobenzene was 7.46 per cent N, as compared with the theoretical for N of 8.89 per cent. The t value of 2.76 again indicates a probability of 0.98 that the deviation from theory was not due to chance.

The fact that twelve analysts obtained values with a mean of 17.87 per cent N gives promise that, by further study, the method can be made satisfactory for some nitro compounds. The choice of p-nitrochlorobenzene was in a sense unfortunate, because in some cases the low results were apparently due to distillation of the sample from solution during reduction. This demonstrated, however, one of the faults of the method. Another and more important objection is that the sample must be soluble in water, alcohol, or acetone.

Since this study was begun, work has been reported by Secor *et al.* (3) on the analyses of N—O and N—N groups by the Friedrich's method. Their data show that the larger amount of potassium sulfate used (1.30 g per 2 ml acid) was a probable source of error in the 1949 study of this method. Therefore, a further collaborative study of a revised method should be made.

RECOMMENDATIONS*

As a result of the data obtained in this year's study, it is recommended that the microkjeldahl method, first action, remain as adopted (1.30 g potassium sulfate per 2 ml sulfuric acid) and that the method be further proved by collaborative analyses of a greater variety of nitrogenous compounds. Also, it is recommended that work on the sodium hyposulfite ($Na_2S_2O_4$) method for reduction of nitro groups be suspended until a further study of the Friedrich's hydriodic acid reduction method has been made.

The appointment of an Associate Referee on methods for micro group analysis is recommended, and also that work be conducted on methods for the halogens, sulfur, and phosphorus.

LITERATURE CITED

(1) HOEL, PAUL G., "Introduction to Mathematical Statistics," p. 71, New York, John Wiley & Sons, Inc. (1947).
(2) OGG, C. L., and WILLITS, C. O., *This Journal*, 33, 100 (1950).

(3) SECOR, GERALDINE E., LONG, MARION C., DUGAN, MARY T., and WHITE, LAWRENCE M., *Ibid.*, **33**, 872 (1950).

(4) SHAEFER, W. E., and BECKER, W. W., *Anal. Chem.*, **19**, 307 (1947).

(5) SNEDECOR, G. W., "Statistical Methods," 4th Ed., p. 82, Ames, Iowa, Iowa State College Press (1946).

(6) ———, *Ibid.*, p. 218.

(7) ——— *Ibid.*, p. 42.

(8) WILLITS, C. O., and OGG, C. L., *This Journal*, **32**, 561 (1949).

(9) ——— *Ibid.*, **33**, 179 (1950).